Effects of altered TatC proteins on protein secretion efficiency via the twin-arginine translocation pathway of Bacillus subtilis

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Table S2. Overview of effects of amino acid substitution in TatC proteins on substrate translocation.

	SufI			TorA			spTorA-fusion			YwbN			PhoD		
	+	+/-	-	+	+/-	-	+	+/-	-	+	+/-	-	+	+/-	-
E.c. TatC															
L9F (N)								x [K]							
H12A (N)				x [A]	x [B]		x [A]								
L13A (N)				x [A]			x [A]								
E15A (N)				x [B]											
E15Q (N)				x [A]			x [A]								
L16A (N)					x [A]			x [A]							
R17A (N)					x [B]	x [A]			x [A]						
R17K (N)				x [B]											
K18M (N)								x [K]							
K18E (N)								x [K]							
R19A (N)				x [B,A]			x [A]								
R19K (N)				x [B]											
L20A (I)					x [A]		x [A]								
N22I (I)								x [K]							
P48A* (P)			x [H]			x [A]			x [A]						
K73A (P)				x [A]			x [A]								
P85A (II)				x [B]											
Q90A (II)				x [B]											
W92A (II)				x [B]											
F94A (II)		x [D]	x [H]			x [B]									
F94L (II)						x [B]									
F94Y (II)				x [B]											
F94S (II)								x [S]							
P97A (C)				x [A]	x [B]		x [A]								
L99A (C)				x [A]	x [B]		x [A]								
L99P (C)								x [S]							
Y100A (C)			x [H]		x [B]										
E103A (C)	x [D]		x [H]						x [B]						
E103D (C)									x [B]						
E103Q (C)				x [A]			x [A]		x [B]						
E103R (C)		x [D]							x [B]						
R104A (C)					x [A]			x [A]							
R105A (C)				x [B]	x [A]			x [A]							
Y126A (III)			x [H]		x [B]										
P142S (P2)								x [S]							
Y154S (P2)					x [A]			x [A]							
E170A (IV)	x [H]				x [B]										
P172A (IV)				x [B]											
L178A (IV)				x [A]			x [A]								
G182A (C2)				x [A]			x [A]								
D211A (V)	x [H,D]								x [B]						
D211E (V)								x [B]							

D211N (V)			x [B]		
S214A (P3)		x [B]			
E227A (VI)		x [B]			
B.s. TatCy					
L11A (N)				x	
H14A (N)					x
I15A (N)				x	
L18A (N)					x
R19A (N)				x	
K20A (N)					x
P96A (C)				x	
G97A (C)				x	
L98A (C)					x
E102A (C)				x	
S108A (C)				x	
B.s. TatCd					
K3A (N)					x
L8A (N)					x
H11A (N)					x
L12A (N)					x
L15A (N)					x
R16A (N)					x
R17A (N)					x
P90A (C)					x
L92A (C)					x
E96A (C)					x
M102A (C)				x	

The effects of site-directed or random mutagenesis in the TatC proteins on the translocation or binding of various Tat-dependent substrates is indicated: +, no effect on protein translocation or binding was observed; +/-, the effect on substrate secretion or binding varied from mild to severe; -, substrate translocation or binding was completely abolished. spTorA-fusion represents several reporter proteins fused to the signal peptide of TorA, such as TorA-GFP (Allen *et al.*, 2002), TorA-MalE (Kreutzenbeck *et al.*, 2007) and TorA-GFP-SsrA (Strauch & Georgiou, 2007). Residues located in the cytoplasmic N-terminus are indicated with (N), in the first transmembrane domain with (I), in the first periplasmic/outer loop with (P), in the second transmembrane domain with (II), in the first cytoplasmic loop with (C), in the third transmembrane domain with (III), in the second periplasmic loop with (P2), in the fourth transmembrane domain with (IV), in the second cytoplasmic loop with (C2), in the fifth transmembrane domain with (V), in the third periplasmic loop with (P3) and in the sixth transmembrane domain with (VI). E.c., *E. coli*; B.s., *B. subtilis*. *, This mutation has been shown to cause disintegration of the TatABC complex (Barrett *et al.*, 2005); K, Kreutzenbeck *et al*., 2007 (random suppressor mutants, substrate translocation); A, Allen *et al*., 2002 (site-directed mutagenesis, substrate translocation); B, Buchanan *et al*., 2002 (site-directed mutagenesis, substrate translocation); D, McDevitt *et al*., 2006 (site-directed mutagenesis, substrate binding); H, Holzapfel *et al*., 2007 (site-directed mutagenesis, substrate translocation and binding); S, Strauch & Georgiou, 2007 (random suppressor mutants, substrate translocation).

REFERENCES

Allen, S. C., Barrett, C. M., Ray, N. & Robinson, C. (2002). Essential cytoplasmic domains in the *Escherichia coli* TatC protein. *J Biol Chem* **277**, 10362–10366.

Barrett, C. M., Mangels, D. & Robinson, C. (2005). Mutations in subunits of the *Escherichia coli* twin-arginine translocase block function via differing effects on translocation activity or tat complex structure. *J Mol Biol* **347**, 453–463.

Buchanan, G., de Leeuw, E., Stanley, N. R., Wexler, M., Berks, B. C., Sargent, F. & Palmer, T. (2002). Functional complexity of the twin-arginine translocase TatC component revealed by site-directed mutagenesis. *Mol Microbiol* **43**, 1457–1470.

Holzapfel, E., Eisner, G., Alami, M., Barrett, C. M. L., Buchanan, G., Lüke, I., Betton, J. M., Robinson, C., Palmer, T. & other authors (2007). The entire N-terminal half of TatC is involved in twin-arginine precursor binding. *Biochemistry* **46**, 2892–2898.

Kreutzenbeck, P., Kröger, C., Lausberg, F., Blaudeck, N., Sprenger, G. A. & Freudl, R. (2007). *Escherichia coli* twin-arginine (Tat) mutant translocases possessing relaxed signal peptide recognition specificities. *J Biol Chem* **282**, 7903–7911.

McDevitt, C. A., Buchanan, G., Sargent, F., Palmer, T. & Berks, B. C. (2006). Subunit composition and in vivo substrate-binding characteristics of *Escherichia coli* Tat protein complexes expressed at native levels. *FEBS J* **273**, 5656–5668.

Strauch, E. M. & Georgiou, G. (2007). *Escherichia coli* tatC mutations that suppress defective twin-arginine transporter signal peptides. *J Mol Biol* **374**, 283–291.